NIH / NIAID Conference: Anti-infective Drug Development

Identification of Small Molecule Inhibitors of Anthrax Lethal Factor

Alan T. Johnson, Ph. D.

02/07/07

PANTHERA BIOPHARMA

Anthrax as a Disease and Weapon

Anthrax (*Bacillus anthracis*) occurs worldwide in soils as a large, spore forming, Gram-positive bacillus.

- In humans, three forms are known; cutaneous, gastrointestinal, and inhalation anthrax.

Classified as a Category A agent by the NIAID.

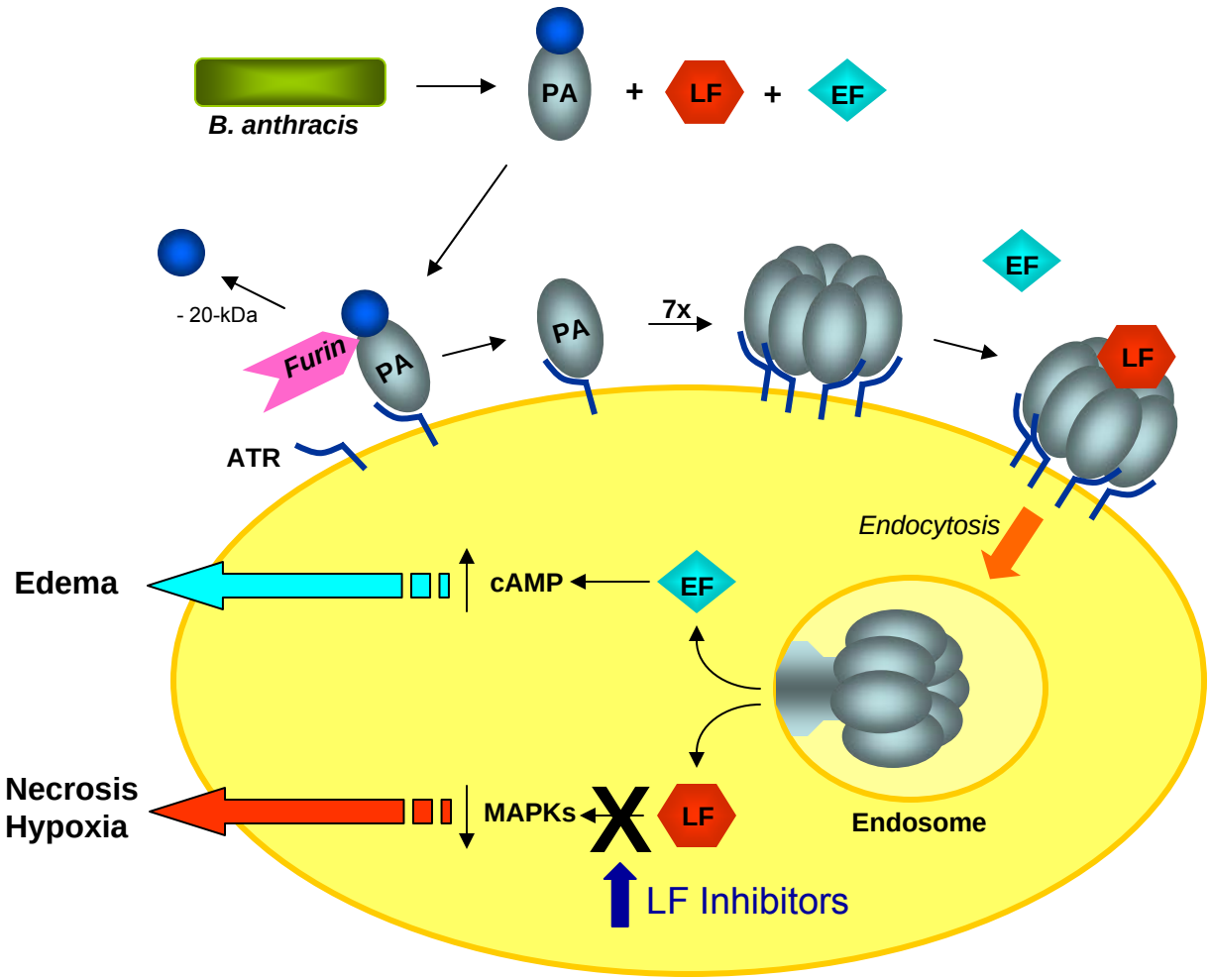
- Anthrax spore attack via US mail (2001) caused 11 confirmed cases of inhalation anthrax resulting in five deaths.

Current therapies include;

- Vaccines such as BioThraxTM (AVA).
- Antibiotics such as Ciprofloxacin which have a narrow therapeutic window.
- Biologics such as ABthrax (vs. PA) which suffer from major logistical drawbacks such as; cost, long term storage, and mode of administration (i.v.).

PANTHERA BIOPHARMA

The Pathogenesis of Anthrax



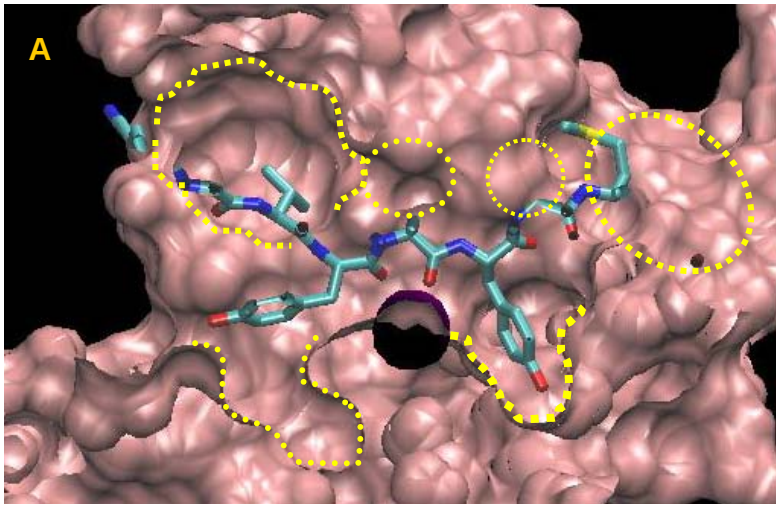
1. Bacteria releases Protective Antigen (PA), Edema Factor (EF), and Lethal Factor (LF). (antibiotics work here)
2. PA binds to cell surface receptor (ABthrax works here)
3. Furin converts PA_{83} to PA_{63}
4. Seven PA proteins combine to provide a door into cell.
5. Heptamer binds EF and LF and undergoes endocytosis.
6. EF and LF are released into cell.
7. EF upregulates cAMP resulting in edema.
8. LF cleaves MAPKs shutting down cell machinery resulting in cell death.

Adapted from Prince, A.S. *J. Clin.Invest.* **2003**, *112*, 656

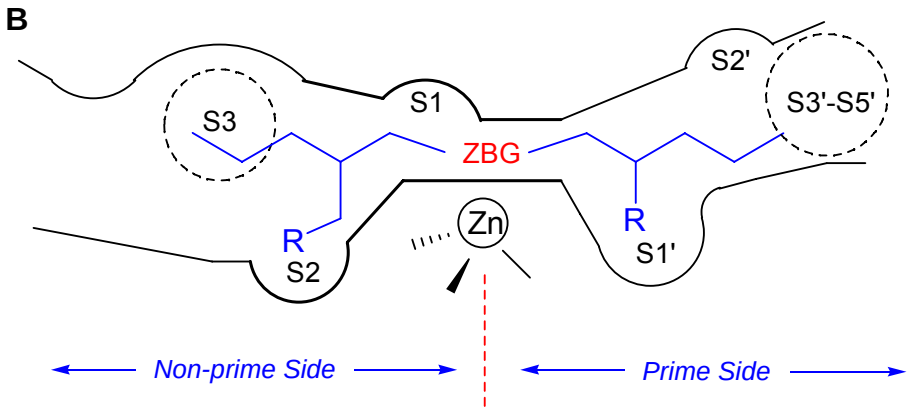
PANTHERA BIOPHARMA

Anthrax Lethal Factor as a Drug Target

Goal: Neutralize the intracellular effects of lethal toxin by inhibiting the LF protease



Cross section of LF Binding Site with bound peptide substrate.



Schematic view of the LF Binding Site

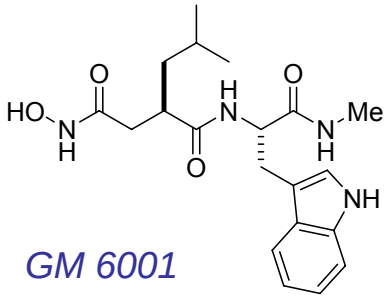
Turk, B.E.; *et al. Nat. Struct. Mol. Biol.* **2004**, *11*, 60

Requirements for good affinity

- A strong Zinc Binding Group (ZBG).
- Occupancy of the S1' subsite.

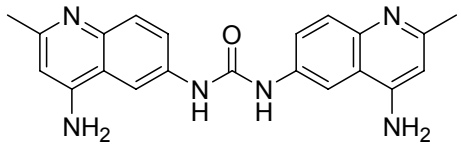
PANTHERA BIOPHARMA

Small Molecule LF Inhibitors



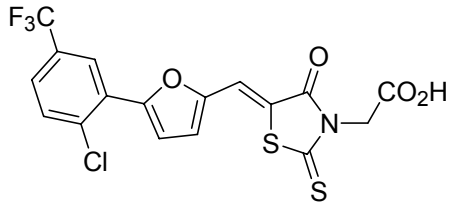
GM 6001

Turk, B.E.; *et al.*
Nat. Struct. Mol. Biol. **2004**, *11*, 60



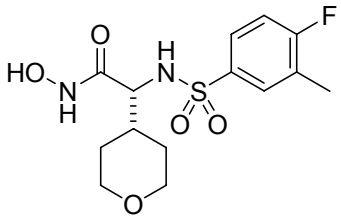
NSC 12155

Panchal, R.G.; *et al.*
Nat. Struct. Mol. Biol. **2004**, *11*, 67



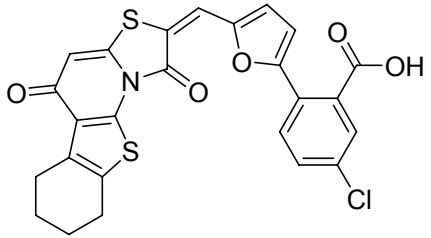
BI – 11B3

Johnson, S.L. *et al.*
J. Med. Chem. **2006**, *49*, 27



Merck L915

Xiong, Y.; *et al.*
Bioorg. Med. Chem. Lett. **2006**, *16*, 964



MSU - 1

Schepetkin, I.A.; *et al.*
J. Med. Chem. **2006**, *49*, 5232

PANTHERA BIOPHARMA

Medicinal Chemistry at PanThera

Computational Chemistry

Molecular Modeling

Virtual screening of chemical databases

- High Throughput Docking (HTD)
- Similarity searching

Synthetic Chemistry

Design and build molecules for testing

- Explore new hit series
- Optimize activity in lead series

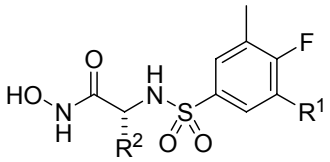
Analytical Chemistry

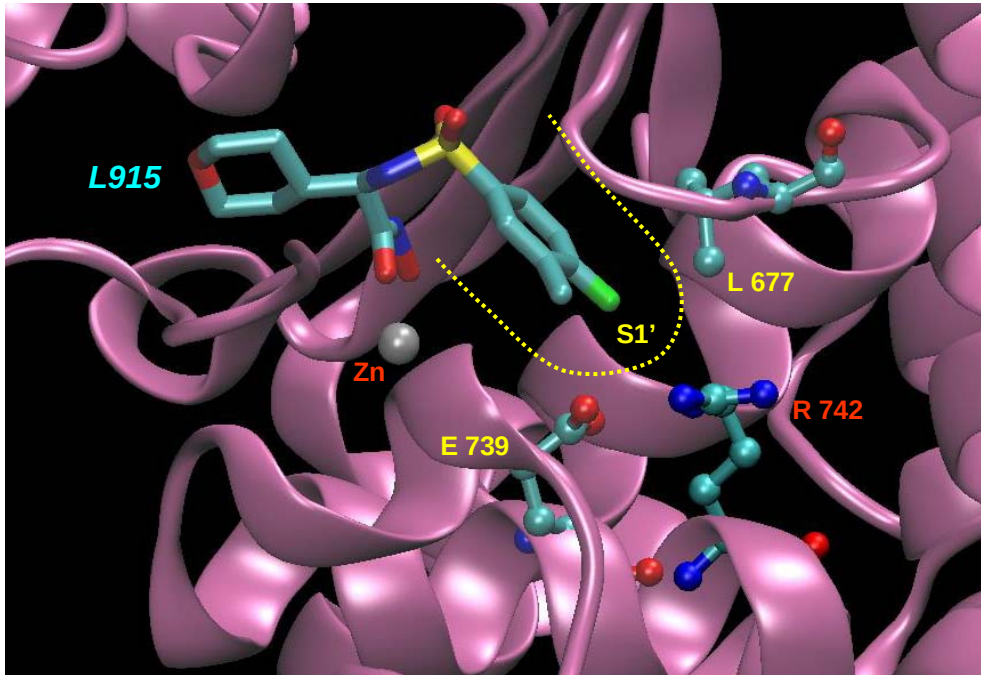
Determine physicochemical properties

- Partition coefficients (ElogD)
- Solubility
- pK_a determinations

PANTHERA BIOPHARMA

LF Mutants as a Tool for Drug Discovery

Compound	R^1	R^2	LF-wt (μM)	L677A (μM)	E739A (μM)
GM6001			3.64	20.30	>300
L915	H	(*R*)-4-THP	0.34	1.04	77.5
	H	(*R*)-*i*-Pr	0.55	7.31	>300
	Me	(*R*)-*i*-Pr	1.61	3.34	206
	H	(*R*)-Ph	0.46	2.00	283
	Me	(*R*)-Ph	0.61	0.86	>300

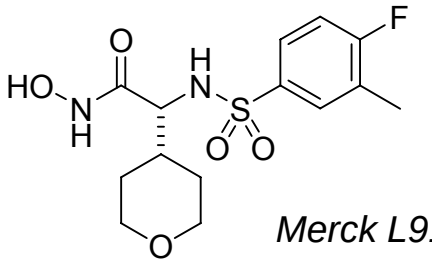


- **Nine LF mutants were made.**
- **Compounds which use S1' for binding to LF will display a large loss in affinity when tested against the E739A mutant.**
- **Structural changes to the S1' binding group can be detected with the L677A mutant.**

Prosise, G.; *et al.* manuscript in preparation

PANTHERA BIOPHARMA

Merck L915: A Benchmark for LF Inhibitors



Merck L915

K_i = 54 nM (PT = 270 nM)
IC_{50} = 210 nM (cell) (PT = 3 uM)

- $t_{1/2}$(h): mouse (0.4), rat (1.4), rabbit (2.0), dog (4.7), monkey (2.4)

- Monotherapy rabbit study: Dose LFI @ 100 mg/kg s.c. t.i.d. for 7 days (*C*max >150 x K_i), Challenge 10^4 spores s. c. Saline controls (n=4), t = 0 (n=4), and t = 24h (n=4).
  Survival rates: saline (0/4), t = 0 (2/4), and t = 24h (1/4).

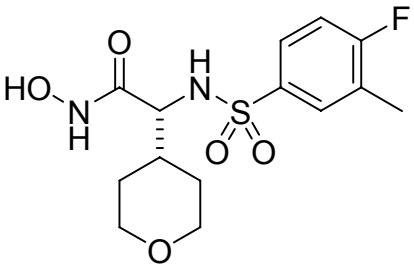
- Cipro and LFI combination therapy: Challenge 10^4 spores s.c. @ t = 0
  Ciprofloxacin @ 5 mg/kg s.c. b.i.d. for 2 days beginning at t = 66h, and LFI @ 100 mg/kg s.c. 4 times a day for 1 day beginning at t = 66h.
  Survival rates: saline (0/3), Ciprofloxin (2/4), Cipro + LFI (4/4).

- The two fatalities in the Cipro group had sterile blood and peritoneal cultures supporting toxemia as the cause of death.

Shoop W. L.; *et al. Proc. Natl. Acad. Sci. USA* **2005**, *102*, 7958.
Xiong, Y.; *et al. Bioorg. Med Chem. Lett.* **2006**, *16*, 964.

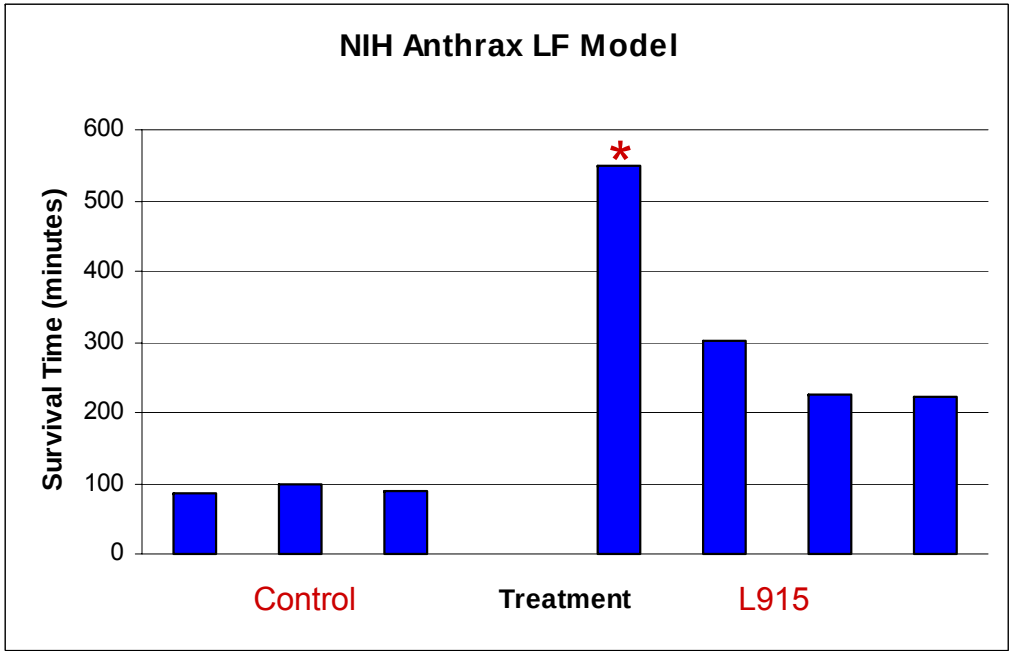
PANTHERA BIOPHARMA

Merck L915 in NIH LT Model Study



Merck L915

LF: K_i^{app} = 0.2 μM
EC_{50}(cell) = 3 μM
$t_{1/2}$ (rat) = 1.4 h



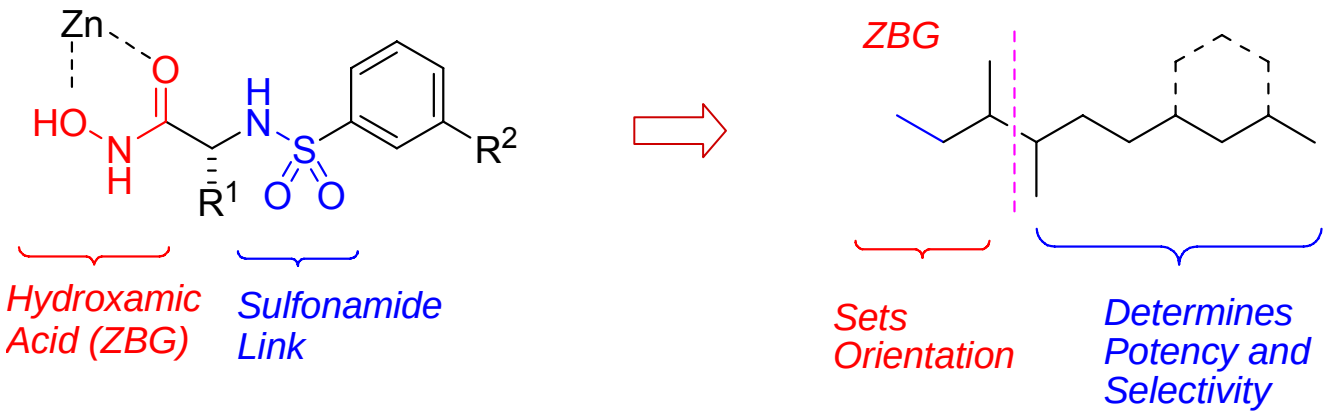
Experimental: Rats dosed at 10 mg/Kg (IV) followed 15 to 18 minutes later by 10 μg LT (IV)

- Mean survival time for control animals was 91 minutes.
- Three of the L915 treated animals survived for 300 minutes or less.
- One animal (*) survived after severe malaise.
- NIH model should be good for comparing new LF inhibitors.

Moayeri, M; *et al. Antimicrobial Agents Chemotherapy*, **2006**, *50*, 2658

PANTHERA BIOPHARMA

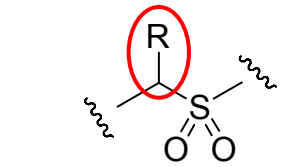
Sulfonamide HAs as a Starting Point



- Core structure size and shape sufficient for binding to LF.
- Modifications possible to ZBG and remaining core structure to improve potency, selectivity for LF, and PK profile.

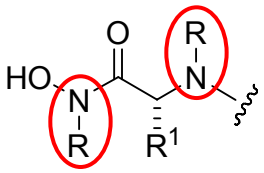
PANTHERA BIOPHARMA

SAR of Aryl Sulfonamide Analogs



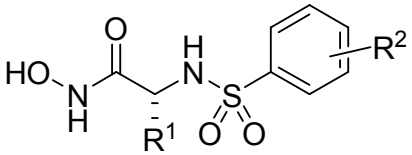
-NH- to –CHR-

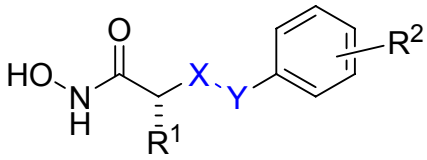
Activity ↑



Additions to core structure

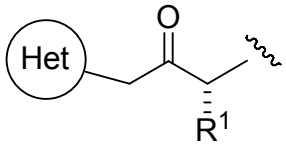
Activity ↓





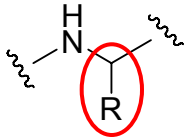
New HA based inhibitors

K_i^{app} < 100 nM



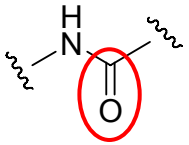
Alternate ZBG

Activity ↓↓



-SO_2 - to –CHR-

Activity ↓



-SO_2 - to –CO-

Activity ↓↓

PANTHERA BIOPHARMA

Acknowledgements

NIH / NIAID (R44 AI052587)

Medicinal Chemistry

Sean O'Malley
Sina Sareth
Seongjin Kim
Guan-Sheng Jiao
Chris Bayne
Linda McKasson
Mellisa Nagata
Ondrej Simo
Cho Tang

Lead Discovery / Biology

Sherri Millis
Lynne Cregar-Hernandez
Zhiliang Wang
Dominique Nguyen
Glen Prosise
Alan McClelland
Mark Goldman
Tom Humphreys

NIH / NIAID

Stephen Leppla
Mahtab Moayeri

Vollum Institute

Gary Thomas
Jason Larson

PANTHERA BIOPHARMA